IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Named Inventor	:	Muriel Pipelier et al.	Confirmation No.: 1347
Appln. No.	:	10/581,226	Group Art Unit: 1625
Filed	:	June 2, 2006	Examiner: David E. Gallis
For	:	NEUROACTIVE SUBSTANCE AND USES OF ONE SUCH SUBSTANCE	
Docket No.	:	U25.12-0001	

DECLARATION UNDER 37 C.F.R. § 1.132

VIA ELECTRONIC FILING

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Muriel Pipelier hereby declare as follows:

1. I am a co-inventor of the subject matter of the above-identified patent application.

2. I am currently "Maitre de Conférences" at "Universite De Nantes", in Nantes, France.

3. My Curriculum Vitae is attached hereto as Exhibit A.

4. I declare that with respect to the structure and conformation of the compounds, the molecular formula $C_{16}H_{24}O_5$ for fulvol acetate isolated from the soft coral Rhytisma fulvum was established by HRESIMS ($[M+Na]^+$ m/z 319.1521, calcd 319.1524, Δ -0.3 mmu) and it indicated 5 degrees of insaturation. Fulvol compound was also isolated from the soft coral Rhytisma fulvum and was used for stereochemistry elucidation of fulvol acetate. The molecular formula $C_{14}H_{22}O_4$ for fulvol isolated from the soft coral Rhytisma fulvum was established by HRFABMS ($[M+H]^+$ m/z 255.1594, calcd 255.1596, Δ -0.2 mmu) and it indicated 5 degrees of insaturation. The relative structures of fulvol and fulvol acetate were solved by their DEPT ^{13}C spectra and by two-dimensional NMR studies (See attached Table 1).

5. I also declare that excellent quality crystals of fulvol were spontaneously obtained. X-ray crystallographic analysis elucidated the relative configuration and fulvol was finally deduced to be 6α-acetyl-4β, 5β-dimethyl-1(10)α-epoxy-2α, 7α-dihydroxy-decalin. Total acetylation of fulvol and fulvol acetate led to the same fully acetylated product (complete matching of the NMR spectra) indicating that fully acetylated derivatives of fulvol and fulvol acetate had the same stereochemistry, it was concluded that accordingly fulvol and fulvol acetate have also the same stereochemistry. This was confirmed by similar [α]20D values in CH_3OH and similar NMR data.

6. I further declare that based on these results, it is reasonable to assume that the fulvol acetate isolated from the soft coral Rhytisma fulvum corresponds to 6α-acetyl-4β, 5β-dimethyl-1(10)α-epoxy-2α-hydroxy-7α-acetoxydecalin (compound of formula II).

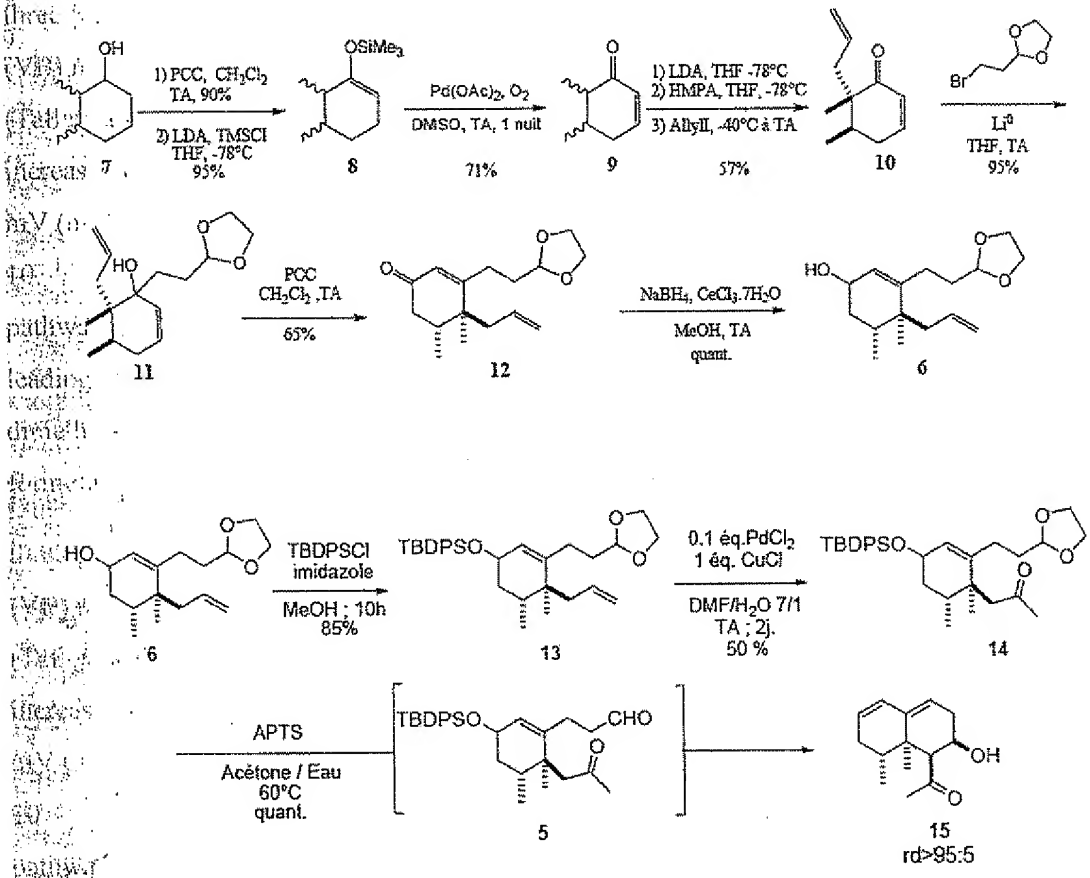
7. I declare that with respect to the neuroactivity of the compounds, the effect of fulvol acetate (compound of formula II) was examined on insect neurosecretory cells named DUM neurons. These DUM neurons are known to display beating spontaneous electrical activity (Grolleau and Lapied 2000; Wicker et al. 2001). DUM neurons were pretreated with 4-AP, a specific blocker of the A-type potassium current known to regulate the firing frequency (Grolleau and Lapied 2000). Under these conditions, the somata of DUM neurons generated a spontaneous firing (about 1-3 Hz in frequency) of action potential (Fig. 1B) Bath application of 100 μM of fulvol acetate produced an important increase in the action potential discharge frequency without any modification of the firing pattern and action potential amplitude but reducing the spike interval and increased the slope of the predepolarization.(Fig. 1C and 1E). This effect was associated with a slight increase of the amplitude of the posthyperpolarization (Fig. 1D).

8. I further declare that additional experiments were performed, under voltage-clamp condition, in order to study the effect of fulvol acetate on the global LVA calcium currents, the maintained component mLVA calcium current and the HVA calcium current. As illustrated in Fig. 2Aa, 2Ac and 2Ba, fulvol acetate only strongly increased the peak total LVA calcium current amplitude without any effect on both mLVA current and HVA calcium current (Fig. 2C and 2F). Fulvol acetate-induced stimulation of current amplitude reached a maximum stable level within about 8 minutes (Fig. 2D).

9. I also declare that in addition, experiments were also performed on native whole-cell T-

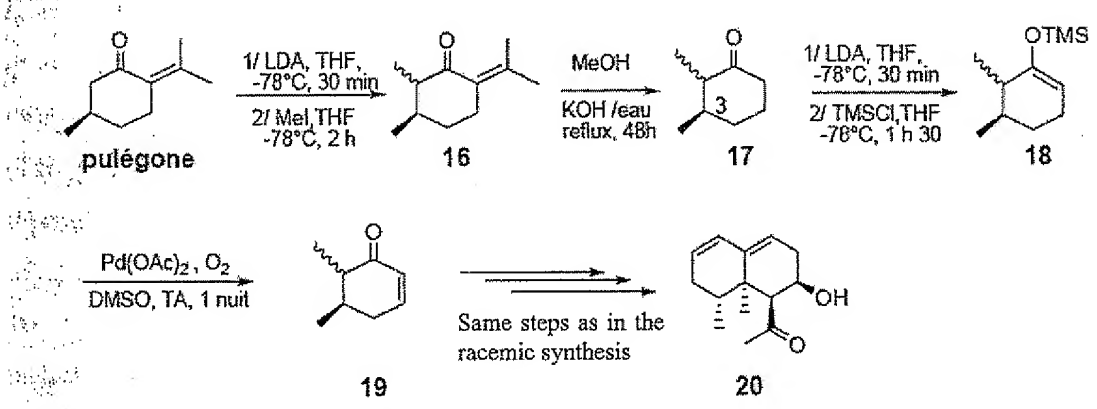
type currents recorded in brain slices in the region of the thalamus containing mRNA for the three fid-fferent isoforms: reticular thalamic nucleus (nRT) (Cav3.2 and Cav3.3), ventro-basal (VB) nucleus (Cav3.1) as well as dorsal root ganglion (DRG) cells containing largely Cav3.2 (Talley et al. 1999). As illustrated in Figures 3C and 3D, external applications of fulvol acetate increased the amplitude of native rat neuronal T-type current elicited by a test-potential to -50 mV (nRT and VB neurons) or -30 mV (DRG neurons) from holding potential of -100 mV.

I declare that with respect to the chemical synthesis of the compounds, the synthetic pathways are depicted below. For racemic synthesis, the different steps of the synthetic pathway leading from the dimethyl-cylohexanol compound 7 to the intermediate 15 (1-(2-hydroxy-8,8a-dimethyl-2,3,7,8,8a,1-hexahydro-naphthalenyl)-ethanone) in the fulvol acetate (compound of formula II) synthetic pathway, are depicted below:



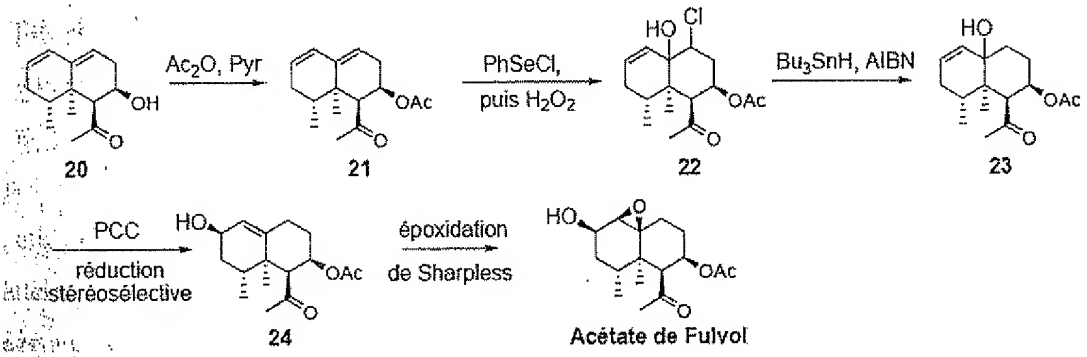
I declare that with respect to pure-enantiomeric synthesis, the different steps of an

alternative synthetic pathway leading from the pulegone compound to a precursor 20 of fulvol acetate (compound of formula II) are depicted below:



Dimethyl-cyclohexanol compound

12. I declare that with respect to synthesis for intermediate 15 or 20 to fulvol acetate (compound of formula II), the final steps of the synthetic pathway leading to fulvol acetate compound are as follows:



13. I declare that all the chemical reactions implemented in theses synthetic pathways are well-known to a person of ordinary skill in the art and that other well-known chemical reactions could be implemented in order to obtain the same intermediates and final compound.

14. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced

application or any patent issuing thereon.

Inventor: Muriel Pipelier
(Printed Name)

Inventor: _____
(Signature)

Date: 1 fev 2010